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ADDITIONAL PHTHALIDE DERIVATIVES FROM *MEUM ATHAMANTICUM*

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In our earlier communications, we have reported the isolation and characterization of cinnamic acid esters (1, 2) and phthalides (3) from *Meum athamanticum* Jacq. (Umbelliferae) rhizomes. We now report the isolation of the seven hydroxylated phthalides listed below and their identification by standard spectral methods. Use of ^{1}H-nmr data of *Z*-3-butylidenephthalide, a common product isolated from the same source, was helpful for analysis of aromatic products. Extraction of the underground parts of *M. athamanticum* with *n*-hexane afforded 7-hydroxy-3-butylidenephthalide; on the other hand, the $CHCl_3$ extract yielded 4-hydroxy-3-butylidenephthalide, 5-hydroxy-3-butylidenephthalide, 3-(2-hydroxybutylidene)-phthalide, 9-hydroxyligustilide, and *cis*- and *trans*-6,7-dihydroxyligustilide. All of these compounds, found in the *Z*-form, are reported for the first time in the genus *Meum*. With the exception of 5-hydroxy- and 4-hydroxy-3-butylidenephthalide, each compound has been described in just one of two other Umbelliferous plants, *Ligusticum wallichii* Franch. [7-hydroxy-3-butylidenephthalide, *cis*- and *trans*-6,7-dihydroxyligustilide (4)], and *Cnidium officinale* Makino [9-hydroxy-3-butylidenephthalide, 3-(2-hydroxy butylidene)phthalide (5)]. 5-Hydroxy-3-butylidenephthalide is present in both of the above species (5, 6).

^{1}H-nmr data ($CDCl_3$) relative to 4-hydroxy-3-butylidenephthalide have been wrongly assigned to the 7-hydroxy isomer in *C. officinale* (5). Correction was made possible as a consequence of the isolation of both 4-hydroxy- and 7-hydroxy derivatives from *M. athamanticum*. The H-8 resonances in the two ^{1}H-nmr spectra, associated with the uv behavior of the related compounds in the presence of $AlCl_3$, clearly distinguished between the two isomers. Effectively, deshielding of H-8 at δ 5.95 ppm is observed when the hydroxyl group is located at the 4-position (γ-relationship), compared with the δ-value recorded at 5.68 ppm for this proton in the 7-hydroxy compound, as in *Z*-3-butylidenephthalide at δ 5.64 ppm. This was also shown and confirmed by the existence of a bathochromic uv shift (λ340 nm$\rightarrow\lambda$375 nm) after addition of $AlCl_3$ for the 7-hydroxy derivative, the 4-hydroxy compound being insensitive. Finally, ^{1}H-nmr and uv records were in agreement with chromatographic data (tlc and hplc), indicating that the 7-hydroxyphthalide was less polar than the 4-hydroxy isomer.

The same observation can be made for 6,7-dihydroxy-3-butylidenephthalide described as the 4,5-dihydroxy isomer in *L. wallichii* (6) since, in this case, H-8 which is not affected by deshielding induced by hydroxylation in the 4-position is recorded at δ 5.54 ppm (CD_3OD).

Finally, on the basis of the reported compounds, the three species, *C. officinale, L. wallichii,* and *M. athamanticum*, collected in the subtribe Seselinae in the family Umbelliferae, likely produce phthalides by the same biosynthetic pathways, probably from *Z*-ligustilide, the most accumulated phthalide in Umbelliferous plants (7).

EXPERIMENTAL

PLANT MATERIAL.—*M. athamanticum* rhizomes were collected from Col du Lautaret, France, at the beginning of the fruiting stage, as previously reported (1-3). A voucher specimen MAR-84 has been deposited at Laboratoire de Pharmacognosie de Grenoble, Domaine de La Merci, F-38700 La Tronche.

EXTRACTION AND ISOLATION OF PHTHALIDES.—The *n*-hexane extract (60 g) was subjected (2 g) to circular centrifugal thin layer chromatography (cctlc) on silica gel GF-254, with $CHCl_3$ as the solvent, affording thirteen fractions. Fraction 1 was used to obtain *Z*-3-butylidenephthalide (12 mg), by repeated cctlc on silica gel with increasing amounts of $CHCl_3$ in *n*-hexane; fraction 4, exhibiting a yellow fluorescence, was chromatographed on a column of polyamide and then purified by cctlc on silica gel with C_6H_6 affording *Z*-7-hydroxy-3-butylidenephthalide (3 mg). The $CHCl_3$ extract (15 g) was fractioned by SiO_2 cc to give nine fractions eluted by $CHCl_3$ up to MeOH. Fraction 2, exhibiting a bluish white fluorescence as with *Z*-ligustilide, was first treated by cctlc on silica gel (*n*-hexane-$CHCl_3$-*i*PrOH-MeOH, 36:2:1:1) and

then by polyamide cc (C_6H_6 up to C_6H_6-MeOH, 95:5) to lead to *Z*-3-(2-hydroxybutylidene)phthalide (1.5 mg) and *Z*-9-hydroxyligustilide (2.5 mg) separated by semi-preparative hplc on Lichrosorb Si 60 (*n*-hexane-*i*PrOH, 98:2) and to *Z*-4-hydroxy-3-butylidenephthalide (4 mg) also purified by hplc but with *n*-hexane-$CHCl_3$-*i*PrOH-MeOH (95:3:1:1). Fraction 4 was directly subjected to the last hplc system to yield *Z*-5-hydroxy-3-butylidenephthalide (14 mg). After filtration through a Sephadex LH 20 column, fraction 7 was first treated by SiO_2 cc and then by cctlc on silica gel (*n*-hexane-$CHCl_3$-*i*PrOH-MeOH, 36:2:1:1) to afford *Z-cis*-6,7-dihydroxyligustilide (13 mg). Fraction 8 was subjected to SiO_2 cc ($CHCl_3$-MeOH, 90:10), then to Sephadex LH 20 (MeOH) to isolate *Z-trans*-6,7-dihydroxyligustilide (11 mg) purified by cctlc on silica gel (*n*-hexane-$CHCl_3$-*i*PrOH-MeOH, 80:10:5:5).

Identification of the isolated phthalides was performed by analysis of their spectral data. Additional details of the spectral properties may be obtained from the senior author.

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FLAVONOLS FROM *GUTIERREZIA ALAMANII* VAR. *MEGALOCEPHALA*

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As part of a chemosystematic study of the *Gutierrezia-Xanthocephalum* complex in North America (1), we previously reported the isolation of 34 flavonoids from *Gutierrzia grandis* (see 2) and 51 from *Gutierrezia microcephala* (3); from a different population of the latter species, other workers reported 21 flavonoids (4). Most compounds of these two woody species have 6,8-oxygenation. Here we report our results from *Gutierrezia alamanii* A. Gray var. *megalocephala* (Fern.) M.A. Lane, a perennial herbaceous plant. Eight flavonols were obtained, namely quercetin, quercitrin, rutin, kaempferol, quercetin-3,7-dimethyl ether, quercetin-3,7,4′-trimethyl ester (ayanin), 5,7-dihydroxy-3,3′,4′,5′-tetramethoxyflavone, and 5,3′,5′-trihydroxy-3,7,4′-trimethoxyflavone. The chemical data support a separation of the herbaceous species of *Gutierrezia* from the woody members that produce 6,8-oxygenated flavonoids. Previously, in 1964, 5,3′,5′-trihydroxy-3,7,4′-trimethoxyflavone was isolated from *Rhicinocarpus stylosus* (Euphorbiaceae) (5); we present spectral data (ms, uv, ^{1}H nmr) of this compound, not included in the earlier report.

EXPERIMENTAL

PLANT MATERIAL.—The aerial parts of *G. alamanii* var. *megalocephala* were collected at Municipio Ciudad Guererro, 32 km N of San Juanito, on the road to Creel, Chihuahua, Mexico, on September 10, 1984. A voucher specimen (Fred R. Barrie & Mark E. Leidig no. 993) is on deposit in the Herbarium of the University of Texas at Austin.

EXTRACTION, ISOLATION AND IDENTIFICATION.—Dried aerial parts of *G. alamanii* var. *megalocephala* (400 g) were extracted three times with 80% and 50% aqueous MeOH. The concentrated extract was partitioned against CH_2Cl_2 and EtOAc. The CH_2Cl_2 fraction was chromatographed over a Polyclar column using a CH_2Cl_2-EtOAc gradient with increasing amounts of EtOAc. The following compounds were sequentially obtained: 5,7-dihydroxy-3,3′,4′,5′-tetramethoxyflavone, quercetin-3,7,4′-trimethyl ether (ayanin), 5,3′,5′-trihydroxy-3,7,4′-trimethoxyflavone, and quercetin-3,7-dimethyl ether. The EtOAc fraction, which was also chromatographed over a Polyclar column using a CH_2Cl_2-MeOH gradient with increasing amounts of MeOH, afforded kaempferol, rutin, quercitrin, and quercetin. All compounds, which were purified over Sephadex LH-20 (100% MeOH) prior to spectral analysis, were identified by uv, ^{1}H nmr, and color reactions (6). Mass spectra were recorded for 5,7-dihydroxy-3,3′,4′,5′-tetramethoxyflavone, quercetin-3,7,4′-trimethyl ether, 5,3′,5′-trihydroxy-3,7,4′-trimethoxyflavone, and quercetin-3,7-dimethyl ether.